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TRANSITION METAL CHELATOR THERAPY - A POTENTIAL TREATMENT FOR ALZHEIMER'S DISEASE?

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1. ABSTRACT

A defining feature of Alzheimer's disease (AD) pathology is the presence of amyloid beta known as A-beta (Aβ within neuritic plaques of the hippocampus and neocortex of the brain. While early *in vitro* studies suggested that Aβ could itself be toxic to neuronal cells, recent studies have indicated that this peptide has both neurotoxic and neuroprotective properties that are modulated by the binding of transition metal ions. Transition metal ion binding was shown to modulate Aβ solubility as well as its hydrogen peroxide production, thereby providing explanations for both its trophic and toxic properties. These findings lead to the suggestion that interference with this interaction may reverse the neurotoxic properties of Aβ. More recently, *in vivo* and *in vitro* studies into the effects of transition metal chelator treatments on Aβ solubilisation and neurological function have been published. Such studies have yielded promising results, however the potential side effects of many such metal chelators may prove too great for clinical use. It is widely agreed that the ideal chelator for such interdiction would act only on those transition metals that complex with Aβ, and only at metal ion binding sites that contribute to Aβ aggregation and reactive oxygen species generation.

The efficacy of metal chelators in reducing Aβ load in transgenic mouse brains demonstrates that this approach has considerable merit as a research tool and as a stimulus to develop second generation agents that can selectively prevent transition metals from binding to the Aβ peptide itself without perturbing the action of other important metal requiring biomolecules in the brain.

2. INTRODUCTION

Alzheimer's disease (AD) is characterised by the presence of neuritic plaques (senile plaques) and neurofibrillary tangles (NFT) in the hippocampus and neocortex of the brain (1,2,3,4). The major protein component of AD

auritic plaques is the small 40-43 amino acid peptide amyloid beta or A-beta (Aβ) (2,3). While a causal link etween Aβ?and the clinical presentation of AD associated dementia has not been proven, a recent study has ovided evidence that Aβ?levels increase in the frontal cortex prior to the formation of NFT's and the onset of ementia (4). Furthermore, Aβ?has shown in many studies, to be inherently toxic to neurons and clonal cell lines culture (5-7).

.1. Aβ functi n as a metallopr tein

)xidative stress is recognised as playing a major role in the pathogenesis of AD (8-9). Aβ can act as an nportant pro-oxidant in this process as it is an electrochemically active metalloprotein with an affinity for ansition metals such as copper and zinc (10-19). Aβ, unlike most physiological metal chelators known to date, ; not a hydrophilic but a lipophilic peptide. The structure of Aβ contains two major sites that may play a role in its nteraction with metals. The first site consists of three histidine and one tyrosine residue in the hydrophilic N-erminal part of the peptide (14-15, 20). These residues are known to efficiently bind transition metal ions, hence ney may contribute towards inhibiting metal-catalysed oxidation of biomolecules (14, 21-22). The second site onsists of a single methionine residue at position 35 in the lipophilic C-terminal region and may act to both cavenge free radicals and reduce metals to their high-active low-valency form (21, 23-27). Aβ has, therefore, een shown to both chelate and reduce metal ions.

The highest binding affinity is observed for copper and is comparable to that of the most efficient metal chelators nown (15). $A\beta_{1-42}$ demonstrates a higher affinity for Cu^{2+} than $A\beta_{1-40}$ and also acts as a more powerful eductant. The highly active reduced metal ions are efficient oxidants and can catalyse further oxidation of iomolecules, for example, through the generation of the highly reactive hydroxyl radical from hydrogen eroxide, a by-product of mitochondrial electron transport and other cellular metabolic reactions (21, 28). It has een demonstrated that this mechanism may play an important role in potentiating the neurotoxic effect of Aβ in ell culture (25, 29).

.2. Metal ion involvement in Aβ related AD pathology

Apart from its neurotoxic properties we have previously demonstrated that Aβ can exhibit pronounced anti-poptotic properties, which, paradoxically, relate to its metal binding capacity and superoxide dismutase (SOD)-ike activity (30). At low, nanomolar, concentrations Aβ was able to significantly reduce apoptotic DNA ragmentation in a corpus luteum organ culture model as well as neuronal cells. Interestingly, $A\beta_{1-42}$ exhibited a nore potent anti-apoptotic action at low doses than $A\beta_{1-40}$. This is likely to reflect its superior copper binding affinity, as the anti-apoptotic effect of Aβ was observed to be dependent on copper binding to the peptide. This inding has recently been supported by the work of Kontush, *et al* (31), who found that Aβ in physiological concentrations (0.1-1.0nM) inhibits autooxidation of cerebrospinal fluid (CSF) lipoproteins and low density ipoprotein (LDL). However, at increased concentrations of 7.5uM and 10uM, we found the resulting $A\beta\text{-}Cu^{2+}$ complex lost it's anti-apoptotic action, and in fact resulted in increased apoptosis (30).

The superoxide dismutase-like activity of Aβ has recently been closely examined by Curtain, *et al* (27) who has proposed a model that incorporates copper and zinc binding to Aβ to generate an allosterically ordered membrane-penetrating structure containing SOD-like subunits. Similarly, Rottkamp and colleagues (32) have postulated that the neurotoxicity associated with transition metal ion binding to Aβ results from lipoxidation. Localized increases in hydrogen peroxide within the lipid bilayer in the vicinity of redox metal ions may explain the increased lipoxidation and neurotoxicity associated with Aβ:Cu complexes, and the inability of the normal antioxidant enzymes to neutralize the hydrogen peroxide produced at high Aβ concentrations.

It has been suggested that pro-oxidant versus antioxidant activity may be dependent on a balance between cellular reductants and antioxidants in the local microenvironment of the senile plaques and NFT (21, 33). A recent study using a novel *in situ* detection system provided evidence for the presence of redox-active transition metal bound in NFT and senile plaques (33). Apart from the potential generation of hydroxyl radicals resulting from an interaction between Aβ and transition metals, other theories for the detrimental effects of transition metals in neurodegenerative disorders have been proposed. One study reports that the formation of advanced glycation endproducts (AGE) as a result of interaction with glucose or fructose and synthetic Aβ is accelerated by

micromolar amounts of coper and iron ions (34). The formation of these high-molecular-mass Aβ oligomers was prevented by capping agents of amino groups, redox-inactive metal chelators and antioxidants. Any mechanism that promotes Aβ aggregation and deposition may be an important contributor to AD pathology. Similarly, another study reports an observation that human Aβ appears to lose histidine residues as the potential result of copper oxidation (16). Modifications of the primary structure of Aβ may lead to changes in its physiological activity. It is conceivable that transition-metal mediated oxidation of the Aβ peptide itself may contribute to the role Aβ plays in AD pathology.

The loss of anti-apoptotic activity and observation of oxidative effects with increasing doses of Aβ (30, 31) is not necessarily in dissonance with the observed protective anti-apototic effect at low doses. In fact, Chan, *et al* (30) proposed a model in which Aβ at low doses complements the actions of intracellular SOD in the brain and may present one reason for increased Aβ production after head injury (35-37). Pathological effects resulting from the SOD like activity of Aβ are likely to occur only if at some threshold level of Aβ the generation of intracellular hydrogen peroxide exceeds the capacity of peroxidases to remove it. This scenario would lead to increased hydroxyl radical formation resulting in cell damage and death (30). This proposition is in accord with findings from numerous studies linking Aβ, copper and other metals to AD and other degenerative disorders (for recent reviews see 32, 38-40). Therefore, the regulation of Aβ interaction with transition metals may represent a valid and successful strategy for therapeutic intervention. Accordingly, a recent focus for AD treatment strategies centres around the principle of depleting levels of transition metal ions which through an interaction with Aβ may lead to oxidative stress.

3. CHELATION AS A POTENTIAL TREATMENT?

Not surprisingly, with the constant stream of new knowledge about transition metal-protein interactions emerging from laboratories all over the world, there has been a rush to utilize this knowledge for the development of new treatment strategies for disorders in which oxidative stress plays an underlying role. In this section we summarize the *in vitro* and *in vivo* studies demonstrating the effectiveness of chelators in the treatment of disorders involving metalloproteases, including AD.

3.1. *In vitro* studies

Given the conclusions drawn from these studies, for the therapeutic dissolution of Aβ from plaques, the most appropriate chelating agents should be relatively selective for Cu^{2+} and possibly Fe^{3+} and Zn^{2+}, but should not sequester Mg^{2+} or Ca^{2+} or other essential trace metals and should be selectively active at the sites of the cerebral amyloid masses (41). Indeed, the laboratory of Bush and colleagues together with our laboratory has successfully demonstrated that the use of metal chelators can solubilize Aβ from post-mortem AD brain tissue (41, 42, 43).

We demonstrated an enhanced solubilisation of Aβ from human and murine brain tissue by the employment of the metal ion chelator TPEN (N,N,N' ,N' -tetrakis(2-pyridylmethyl) ethylenediamine) and the chelator/antioxidant 1,2-dithiolane-3-pentanoic acid (α-lipoic acid) in a dose dependant manner (43) (Figure 1). Clinical and experimental studies have already shown that the powerful antioxidant action of α-lipoic acid may be of benefit in ameliorating diabetic pathologies (44). These studies have provided evidence that chelators can disrupt the interaction of transition metal ions with Aβ and demonstrate the potential therapeutic role of metal chelators in the treatment of AD. Interestingly, a very recent study has provided evidence to show that AGE inhibitors may assert their activity primarily through their chelating or antioxidative properties rather than their carbonyl trapping activity (45). This is significant, as it suggests that removal of metal ions could potentially have wider reaching beneficial effects than just the abolishment of their direct interaction with the Aβ peptide.

3.2. *In vivo* studies

3.2.1 Animal

A number of studies support the use of metal chelators for therapeutic intervention in AD. A study utilising New Zealand white rabbits showed that desferrioxamine (DFO), a chelator with affinities for aluminium, iron and

copper, could largely prevent the neurotoxic effects of intracerebral injections of aluminium and even partially reverse neurofibrillary degeneration (46). Further work by this group showed that the extensive neurofibrillary degeneration observed in rabbits tr ated with aluminium was immunoreactive for a number of antibodies that stain NFT confirming the relevance of this model to AD pathology (47). A decade earlier it had been demonstrated that DFO could enter the small intestinal mucosa, bind intracellular iron, and block the absorption of inorganic iron, tranferrin iron and haemoglobin iron in both a rat model and human volunteers, thus indicating that DFO treatment may be useful for iron depletion, in the case where aluminium would not be considered to make a significant contribution to AD pathology (48).

Other compounds which have resulted in significantly increased secretion of iron in the mouse, rat or rabbit models include orally active alpha-ketohydroxy puridine iron chelators such as 1,2-dimethyl-3-hydroxy pyrid-4-one (L1) and 1-ethyl-2-methyl-3-hydroxy pyrid-4-one (L1-NEt) (49, 50) as well as phenolic ethylenediamine derivatives including N,N' -ethylene-bis(o-hydroxyphenylglycine) (EHPG), N,N' -Bis(o-hydroxybenzyl)-ethylenediamine diacetic acid (HBED), and their respective dimethyl esters (dmEHPG and dmHBED) (51).

Most recently, a study using a transgenic mouse model of amyoloidosis found a 49% decrease in brain Aβ deposition in a blinded study of AβPP2576 transgenic mice (a model for AD) treated orally with the bioavailable Cu/Zn chelator, Clioquinol. No change in APP, synaptophysin or glial fibrillary acidic protein was observed suggesting that the treatment did not result in obvious major disturbances of the brain cellular environment (42). This study supports the role of metal ion interactions with Aβ in the pathophysiology of AD.

3.2.2 Human

An early study with rhodotorulic acid (RA) showed effectiveness in both animals and humans but human subjects experienced local inflammatory reactions at sites of intramuscular and subcutaneous injections. An increased excretion of zinc caused the authors to suggest that RA may be best used as a second line drug (52). Over a decade ago, a two year, single-blind study investigated the progression of dementia after administration of desferrioxamine (DFO) (53). The hypothesis behind the study was that DFO may reverse dementia induced by aluminium toxicity. Twice daily intramuscular injections of DFO were administered to a randomly assigned test group selected from 48 AD patients. A control group was given a lecithin placebo, while a further control group received no treatment. While no differences in the rate of deterioration were observed between the two control groups over the 24 month period, the DFO treatment resulted in a significant reduction in the rate of decline of daily living skills in the test group (53). This led the authors to conclude that sustained administration of DFO may, indeed, be a useful approach to slow down the clinical progression of AD dementia.

More recently, as a result of the studies discussed in the above sections, focus has shifted towards the removal of copper from the brain. Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline), a chelator that can cross the blood brain barrier and is specific for certain transition metal ions, including copper and zinc, was tested in 20 AD patients (54). It was administered for 21 days at doses of 20 mg/day to 10 patients and 80 mg/day to the remaining 10 patients. This was an open study, blind to the dosages but not including controls. An observation was made that after day seven, cerebrospinal (CSF) Tau protein and growth associated protein (GAP43) were elevated, but decreased again at day 21. It was proposed that the rise and fall in the CSF levels of these proteins may have been due to an increased release into the CSF from tissue stores such as senile plaques. This was substantiated by the finding that CSF-Tau protein levels correlated positively and significantly with the serum copper levels and serum copper/zinc ratio. Furthermore, individuals showed significant improvement in certain cognitive functions after 21 days of treatment with clioquinol. However, the effectiveness of the treatment remains to be determined in future trials in which it would be advisable to include control groups and extend the duration of treatment (54).

4. Important considerations in chelator therapy

When removing metal ions from the body there are some obvious considerations that must be taken into account to protect the individual being treated. If the levels of certain metals are decreased excessively below recommended levels, severe physiological side effects may result. It is also important to identify the most important metal(s) involved in the pathology and to find chelators specific for those metal ions. Ideally, the depletion of metals should be localised to the site of pathology without a systemic depletion of metal ion concentrations. Finally, a prerequisite for successful treatment with any chelator will be the requirement for low toxicity and minimum side effects of the drug itself.

The candidate chelators discussed in this review have been chosen because they are considered to meet the above-mentioned criteria to appreciable degrees. However, there remains room for the development of better alternatives. Clioquinol, for example, which was originally used as an antimicrobial agent, has been shown to produce severe side effects in the central nervous system (CNS) if taken in chronic high doses. As a result of the chronic high dose usage of clioquinol, perhaps coupled with a low B12 status, individuals presented with subacute myelo-optic neuropathy (SMON). This disorder is characterized by peripheral neuropathy and blindness that affected 10,000 patients in Japan, and resulted in the drug being removed from the market. It has recently been reported that the toxicity of clioquinol may have been a direct result of its binding to zinc to form a cytotoxic zinc chelate with a primary effect on mitochondria (55). This illustrates the importance of establishing not only a low toxicity of the drug considered for treatment but also for potential complexes it may form after administration. Furthermore, it appears that clioquinol may affect the homeostasis of vitamin B_{12} in the brain (56, 57). The mechanism of action, however, does not appear to occur through a sequestering of cobalt ions by clioquinol. Rather, a decrease of vitamin B_{12} levels in the blood and the brain was observed in concord with a decrease of S-adenosylmethionine (SAM) levels (56, 57). Therefore, clioquinol may have additional side effects apart from those related to its known chelating activity for certain metals. Hence it would be wise to thoroughly investigate the full spectrum of activity of this drug and conduct longitudinal trials of both its safety and effectiveness, before considering it as a first line treatment option.

Further evidence of the need for ligand-specific metal ion chelators for reversal of AD neuropathology is presented by three recent studies on the role of zinc in Aβ aggregation and toxicity. Studies by Atwood and colleagues (15) demonstrated that there was an antagonistic relationship between Cu^{2+} and Zn^{2+} binding to Aβ that was modulated by pH. In this connection, Suzuki, *et al* (58) recently reported that Cu^{2+} competes with Zn^{2+} for binding to Aβ, thereby inhibiting Zn^{2+}-induced Aβ aggregation. In addition, Cuajungco, *et al* (29) demonstrated that co-incubation of redox-inert Zn^{2+} with Aβ and Cu^{2+} rescued primary cortical and human embryonic kidney 293 cells from the hydrogen peroxide induced damage resulting from Cu^{2+}: Aβ interactions. Therefore while Zn^{2+} may be capable of inducing Aβ aggregation, the above findings suggest a protective role for Zn^{2+} in the presence of copper. Cuajungco and colleagues propose a model of redox-silencing through entombment of Aβ by zinc, contrary to their earlier hypothesis (10). These results further suggest that chelators that are not specific for copper (or iron or aluminium) but also bind zinc, may reduce the protective effects of Zn^{2+}, thereby defeating their intended purpose and potentially inducing harmful side effects.

Apart from considering only the chelators themselves, it is also important to pay attention to other factors involved in oxidative stress production. It has been demonstrated that increased copper and/or homocysteine levels could promote significant oxidative damage to neurons, as homocysteine was found to reduce Cu^{2+} more effectively than cysteine or methionine and was shown to promote Aβ/Cu-mediated hydrogen peroxide production and neurotoxicity (59). This implies that a treatment strategy designed to control levels of metals as well as levels of homocysteine might be more successful than metal chelator treatment alone. It is also important to take advantage of chelators that act through more than just one mechanism. The compound bathocuproine, for example, was found to interact with Aβ to form a complex independent of the presence of copper, as well as being able to chelate copper, and thus contribute to solubilizing Aβ through a dual mechanism of action (60). Likewise, α-lipoic acid has both chelator properties desirable for Aβ solubilisation (43) and antioxidant properties (61). A successful treatment strategy may well involve a combination of several approaches for both the solubilisation and inhibition of redox properties of Aβ in AD brain tissue.

5. PERSPECTIVE AND FUTURE DIRECTIONS

Human trials are already being conducted to evaluate the usefulness of transition metal chelators in the therapy of AD. The most important principles to remember for future development of therapeutic chelating agents are: The agent should be effective in chelating metals such as copper, aluminium (and possibly iron) but should not deplete the CNS or the body of zinc, calcium or magnesium. Careful administration of the agent should ensure that the normal physiological levels of any trace metal is not compromised. An agent that is site specific and depletes metals in the microenvironment where the pathology is located is likely to produce less wide spread side effects than one that acts systemically. In this respect, the agent must be hydrophobic in order to cross the blood-brain-barrier. Most importantly, any agent used for sustained treatment over longer periods of time must have no severe or cumulative detrimental effects for the patient.

It is likely that the experts in the field will pursue different avenues. Some valid approaches may include carefully monitored and strictly controlled administration of agents with similar properties to clioquinol. Combination therapies may be developed in order to obtain optimum reduction of oxidative stress, such as simultaneous treatment with chelators and antioxidants, or the use of chelator antioxidants such as α-lipoic acid. Combination therapies may also be required for assisting in the clearance of the toxic Aβ solubilised from plaques and smaller aggregates. Compounds such as bathocuproine with multiple actions on Aβ solubility may prove beneficial in this regard but must await further evaluation.

Our current knowledge about Aβ aggregation and plaque formation, however, has allowed us to speculate on the optimum characteristics of an agent required for specific dissolution of amyloid plaques in AD brains without adverse effects. Therefore, it will be our goal to find or develop an agent that will block the metal binding capacity of Aβ, without affecting intra- and extracellular metal ion concentrations. In any case, results from current trials will soon reveal the future prospects for metal chelators and related compounds in the treatment o neurodegenerative disorders such as AD.

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ELSEVIER FULL-TEXT ARTICLE

Protection by the heavy metal chelator N,N,N',N'-tetrakis (2-pyridylmethyl) ethylenediamine (TPEN) against the lethal action of botulinum neurotoxin A and B.

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The ability of N,N,N',N'-tetrakis (2-pyridylmethyl)-ethyenediamine (TPEN) to protect against botulinum neurotoxin (BoNT) A and B was examined in vivo in mice. To determine the protective efficacy of TPEN, mice were injected i.p. with TPEN as a single bolus or as multiple injections 30 min before and 0, 2, 4 and 6 hr following i.v. challenges with BoNT-A or -B. TPEN treatment did not alter the 24 hr lethality of BoNT but did produce a significant delay in the time to death. For a moderate dose of serotype A (20 LD50), five divided doses of TPEN prolonged the time to death from 7.8 +/- 0.4 hr to 9.9 +/- 0.5 hr. For serotype B, examined under comparable conditions, the prolongation of the time to death was from 6.1 +/- 0.2 hr to 9.4 +/- 0.6 hr. The range of TPEN doses that could be examined in vivo was limited by its acute toxicity. Although low doses of TPEN (< or = 10 mg/kg) were well tolerated, higher doses (> or = 30 mg/kg) led to ataxia, loss of coordination, convulsions and death in 20.3 min or less. In clonal NG108-15 cells, TPEN was found to produce cytotoxicity as revealed by increases in the secretion of the marker enzyme lactate dehydrogenase (LDH), and enhanced reactivity with the vital dye trypan blue. From LDH concentration-response data determined 24 hr after addition of TPEN, the threshold concentration for observing cytotoxicity was 10 microM and the IC50 was 19.8 microM. At the highest TPEN concentration tested (100 microM), cytotoxicity was detected 8 hr after TPEN addition and increased in severity over a 3 day period. The cytotoxicity in NG108-15 cells appears to be distinct from the rapid-onset toxicity observed in whole animals. These results suggest that TPEN may be of potential benefit in delaying the lethal actions of BoNT-A and -B, but its use is limited by its initial and delayed toxicity. Since the therapeutic and toxic actions of TPEN are both related to zinc chelation, the use of TPEN would need to be restricted to low doses as part of a combination therapy.

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